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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Sette *et al.*

Serial No. 08/452,843

Filed: May 30, 1995

For: HLA BINDING PEPTIDES AND THEIR USES

Examiner: Thomas Cunningham, Ph.D.

Art Unit: 1644

STATEMENT AND PETITION TO MAKE SPECIAL UNDER 37 C.F.R. § 1.102

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants hereby respectfully petition to make special the above-referenced patent application, as an invention relating to either HIV/AIDS or cancer according to MPEP § 708.02(X). Also provided is a statement explaining how the invention contributes to the treatment and prevention of both cancer and AIDS.

Please charge $130.00, pursuant to 37 C.F.R. § 1.17(i), to Deposit Account 20-1430. Please charge any additional fees or credit overpayment to the above Deposit Account. This petition is submitted in triplicate.

To facilitate the discussion of the requirements for granting the petition, submitted herewith is Appendix 1, Celis *et al., Epitope Selection and Development of Peptide Based Vaccines to Treat Cancer, Cancer Biology* 6:329-336 (1995).

REMARKS

1. The invention

The present invention relates to cytotoxic T lymphocytes ("CTLs"). CTLs have the ability to recognize and destroy cells that express certain "foreign" or "abnormal" antigens, e.g., viral and tumor antigens. This CTL response is particularly important in tumor rejection and in fighting viral infections such as HIV. CTLs recognize cells expressing such abnormal antigens because on their surfaces the cells present peptide epitopes derived from the antigens. Such peptide epitopes are presented bound to MHC class I molecules on the surface of cells.

In the general population, numerous alleles encode MHC class I molecules. MHC class I alleles are extremely polymorphic and different MHC class I allele-specific molecules are predicted to bind to different sets of peptides. Peptide binding to a particular MHC class I molecule (encoded by a particular MHC class I allele) thus depends on the sequence of the peptide. These alleles are also grouped into sets of related alleles called "supertypes."

The inventors of the present application have identified for the first time "binding motifs" of conserved amino acids, the presence or absence of which determine peptide binding to a MHC class I allele-specific molecule. When the binding motif is correlated to a supertype of MHC class I molecules, it is referred to as a "supermotif." These binding motifs and supermotifs can therefore be used to identify peptide epitopes and/or prepare peptide epitope vaccines, for inducing a CTL response against a particular antigen.

2. Application to cancer and HIV/AIDS

The cellular immune response is critical for the treatment and prevention of cancer and viral diseases such as HIV infection. Both viral clearance and tumor regression have been shown to be associated with a strong CTL response in patients. Peptide epitopes having a sequence corresponding to a binding motif or supermotif of the invention are therefore used to make disease specific vaccines, which induce CTL responses.

For production of cancer specific vaccines, many different tumor associated antigens have been identified, e.g., CEA, PSA, Her2/Neu, p53, and MAGE. These tumor

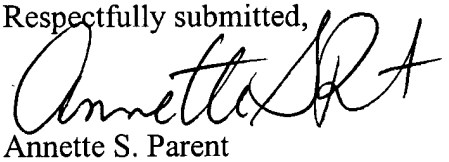
associated antigens are screened to identify motif-bearing epitopes. The epitopes can then be used to make peptide epitope vaccines that target cancers having the tumor associated antigens, e.g., melanoma, breast cancer, lung cancer, colorectal cancer, or prostate cancer (*see* Appendix 1, Celis *et al., Epitope Selection and Development of Peptide Based Vaccines to Treat Cancer, Cancer Biology* 6:329-336 (1995)). In addition, HIV and HPV are examples of well-characterized infectious viral agents. These viral antigens can be used to make peptide vaccines that target HIV infected cells and HPV infected cells associated with cervical cancer.

Thus, an important factor in the efficacy of such epitope-containing vaccines is the ability of the epitopes to bind and be presented by MHC class I molecules. A further factor relevant to efficacy is the ability to bind MHC molecules encoded by alleles that are present at varying frequencies in the human population. To achieve broad population coverage, peptide epitope vaccines should be designed to have a combination of sequences corresponding to various allele-specific binding motifs or supermotifs of the invention. This strategy ensures that the peptides are bound and displayed by genetically diverse MHC class I molecules. The present invention, which provides binding motifs and supermotifs critical for the efficacy of peptide epitope vaccines, therefore makes an important contribution to the treatment and prevention of cancer and HIV infection.

CONCLUSION

In view of the foregoing statement establishing that the present invention contributes to the treatment and prevention of both HIV/AIDS and cancer, Applicants respectfully request that this petition be granted. If a telephone conference would expedite consideration of this matter in any way, please call the undersigned at 415-576-0200.

Respectfully submitted,



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Appendix 1

seminars in CANCER BIOLOGY, Vol 6, 1995: pp 329–336

Epitope selection and development of peptide based vaccines to treat cancer

*Esteban Celis, Alessandro Sette and Howard M. Grey**

Cytotoxic T lymphocytes recognize peptides that associate with class I major histocompatibility complex molecules. Since cytotoxic T cells have the capacity to recognize and destroy tumor cells, identification of epitopes recognized by these cells in tumor-associated antigens would allow the production of compounds for the treatment of cancer. Here we review some of the approaches being explored to identify tumor-associated antigens and to develop peptide-based vaccines that induce cytotoxic T lymphocytes against specific tumors.

Key words: peptide vaccine / tumor-associated CTL epitopes/HLA-binding peptides/cytotoxic T-cell epitopes/ tumor-associated antigens

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THERE IS AMPLE EVIDENCE both in humans and animals that the immune system has the ability to recognize and eliminate tumors. Specifically, cytotoxic T lymphocytes (CTL) are capable of destroying already established tumors, as well as preventing their establishment.[1-3] Here we shall review: (1) some of the molecular events involved in the recognition and elimination of tumor cells by CTL; (2) strategies for identifying tumor-associated antigens (TAA); (3) CTL epitopes on these TAA; and (4) the use of this information for vaccine development.

Antigen recognition by CTL

CTL are characterized by expression of CD8 cell surface molecules and T-cell receptors for antigen (TcR).[4] The TcR of CTL bind to a molecular complex on the surface of the antigen-presenting cells (APC) formed by a peptide epitope usually derived from a viral or a tumor-associated antigen (TAA) and major histocompatibility gene complex (MHC) class I molecules. The peptides that are recognized by $CD8^+$ CTL are usually fragments 8–10 residues long that associate non-covalently with polymorphic class I MHC molecules.[5] Many normal (or abnormal) cellular components as well as proteins derived from genes of foreign intracellular microorganisms can be processed into potential MHC-binding peptides which are transported to the APC surface for presentation to the TcR. After TcR engagement by appropriate MHC-peptide complexes, CTL have the ability to bind and kill target cells expressing foreign (infectious) or tumor-specific antigens.

Some of the changes that occur during cell transformation could create MHC-binding peptides which could potentially be immunogenic for CTL. These include: (1) production of oncogenic viral proteins; (2) abnormal overexpression of fetal or tissue specific proteins; and (3) mutated or overexpressed oncogene or tumor suppressor gene products.[6-10] In the following sections we will review some of the novel methodologies to identify TAA and to define those CTL epitopes from such TAA which can serve as a basis for the development of specific immunotherapeutics for cancer.

Strategies to identify TAA

Over several decades many different TAA such as CEA, PSA, $p185^{HER-2}$ and p53, which serve as 'tumor markers' have been identified and some have been biochemically characterized.[6,11] Because many of these were identified serologically or genetically, their relevance to CTL immunity is unclear. Two new approaches based on advanced molecular biology and immunology techniques made it possible in the last few years to identify several additional protein antigens that can function as TAA for CTL. These approaches are: (1) expression cloning of genes coding for TAA, and (2) elution and direct sequencing of TAA-derived peptides bound to MHC molecules purified from tumor cells. Both approaches rely on the availability of tumor-reactive CTL obtained

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Table 1. Known non-viral TAA and corresponding epitopes recognized by TIL or CTL

TAA	Epitope sequence	MHC restriction	IC^{50} (nM)*	Refs
MAGE-1	EADPTGHSY	HLA-A1	45	13
MAGE-1	SAYGEPRKL	HLA-Cw16	ND	14
MAGE-3	EVDPIGHLY	HLA-A1	4.6	43, 46
MAGE-3	FLWGPRALV	HLA-A2.1	61	44
MART1/Melan-A	AAGIGILTV	HLA-A2.1	150	17, 26
MART1/Melan-A	ILTVILGVL	HLA-A2.1	381	30
pmel117/gp100	KTWGQYWQV	HLA-A2.1	11	18
pmel117/gp100	ITDQVPFSV	HLA-A2.1	84	18
pmel117/gp100	YLEPGPVTA	HLA-A2.1	95	18, 29
pmel117/gp100	LLDGTATLRL	HLA-A2.1	483	18
pmel117/gp100	VLYTYGSFSV	HLA-A2.1	13	18
Tyrosinase	MLLAVLYCL	HLA-A2.1	333	19
Tyrosinase	YMNGTMSQV	HLA-A2.1	40	19
Tyrosinase	AFLPWHRLF	HLA-A24	ND	27
gp75	(unknown)	HLA-A31	ND	20
p15	AYGLDFYL	HLA-A24	ND	21
BAGE	AARAVFLAL	HLA-A2.1	ND	22
GAGE	YRPRPRRY	HLA-Cw6	ND	25
LB33-B	EEKLIVVLF†	HLA-B44	ND	24
CDK4	ACDPHSGHFV†	HLA-A2.1	ND	23
$p185^{HER}$	IISAVVGILL‡	HLA-A2.1	417	9
$p185^{HER}$	KIFGSLAFL	HLA-A2.1	33	10
CEA	YLSGANLNL	HLA-A2.1	14	52

*MHC binding affinity as defined in the text; ND, not done.
†Products of mutated genes; underlined letters represent the mutated residues.
‡Peptide IISAVVGIL (which lack an L at position 10) was described as the CTL epitope, but in our hands it did not bind to HLA-A2.1 $IC^{50} \geq$ 10,000 nM.

from tumor bearing patients to screen gene libraries or peptide fraction isolates.

Expression gene cloning of TAA

Thierry Boon and collaborators in Brussels pioneered the identification of TAA encoding genes of non-viral origin, originally in murine model systems and later in human melanomas.[7] Using chemical mutagens several tumor variant lines were isolated which were rejected by syngeneic mice, suggesting that the mutagen had induced expression of new TAA which involved a CTL response, finally resulting in rejection of the tumor challenge. The genes coding for TAA in various mouse (and later human) tumor variants were identified by transfection of genomic DNA cosmid libraries into recipient cells resistant to lysis by CTL (and thus, not expressing these antigens). By screening the resulting transfected cells for lysis by the TAA-specific CTL clones the gene coding for the TAA was identified. The same approach was used to identify a family of genes expressed predominantly in human melanomas (but also in a small proportion of breast, lung and colon carcinomas) and not in most normal tissues (with exception of the testes) designated *MAGE*.[12] By combined use of fragments of the 3rd exon of *MAGE-1* and synthetic peptides, two epitopes recognized by a melanoma patient's CTL were defined as 9-amino acid peptides which were presented to the TcR in association with HLA-A1 and HLA-Cw16 class I MHC molecules (Table 1).[13,14]

Several additional TAA (MART1/Melan-A; pmel17/gp100, tyrosinase, gp75, p15, *BAGE*, *GAGE*, and others), also expressed mainly in melanomas, were recently identified by the same methodology[15-27] (Table 1). These proteins in addition to being expressed in melanomas, are also found in normal melanocytes. These observations demonstrate that under some circumstances TAA can be derived from normal cell constituents, and that immune tolerance to 'self-antigens' at the CTL level is not necessarily complete.[28] However, in some cases the CTL recognized products of single-point mutations of the normal melanocyte genes, explaining the lack of immune tolerance to these particular epitopes.[23,24]

Elution of TAA peptides

The second approach to identify TAA is to directly sequence MHC-binding peptides eluted from tumor cells. This technique requires large numbers of tumor cells from which MHC molecules can be purified

together with accurate and sensitive methods to characterize the eluted peptides and as in the previous method, TAA-reactive CTL are required to identify the active peptide fractions. This method of antigen identification was first applied successfully in the melanoma system by combined use of microcapillary HPLC and tandem mass spectrometry.[29] A pmel17/gp100 derived epitope and another one from MART-1 recognized by CTL lines derived from melanoma patients have been identified[29,30] (Table 1). Another application of this method was recently described in the identification of the human minor histocompatibility antigen, HA-2.[31] Because the HA-2 antigen is uniquely expressed on hematopoietic-derived cells, HA-2 immunization of bone marrow donors before transplantation into leukemia patients could induce a graft versus leukemia CTL response, thus reducing the risk of leukemia recurrence.

In both these cases, sequences of the genes encoding for the CTL epitopes identified were already known. If, however, epitopes identified using this method were to be derived from unknown sequences, the epitope sequences could be used to clone the corresponding genes by methods such as, for example, anchored DNA polymerase chain reaction.

Identification of CTL epitopes

The two methods described above use CTL isolated from tumor-bearing individuals as probes to identify TAA. In other instances potential TAA can be identified from knowledge of unique association between certain tumors and (1) the presence of viral genes in the tumor (e.g. cervical carcinoma and human papillomavirus (HPV) or Burkitt's lymphoma and Epstein-Barr virus (EBV)[32,33]); (2) the presence of unique tissue-specific proteins that could serve as surrogate TAA (e.g. PSA and PAP for prostate cancer[11]); or (3) the overproduction of normal cellular proteins in certain cancers (e.g. $p185^{HER-2}$ in breast and ovarian cancers or p53 for several types of cancer[5]). Several CTL epitopes designated by the late membrane antigens of EBV have been identified in Burkitt's lymphoma, Hodgkin's Disease and nasopharyngeal carcinoma.[34,35] Comparable HPV designated CTL epitopes are described below. In the case of $p185^{HER-2}$, at least two HLA-A2.1-restricted epitopes have been identified in CTL from patients with ovarian cancer.[9,10] As for the role of the other non-viral proteins as TAA in humans, this will have to be substantiated.

Whatever the method utilized to identify a TAA, for the peptide-based vaccine approach to be broadly applicable it will be necessary to identify multiple peptide epitopes that are presented by all the major MHC class alleles to provide broad population coverage and to prevent tumor escape by mutation of a single CTL epitope. In our laboratory we have developed a strategy to identify peptide epitopes for CTL from putative or known TAA which involves three critical steps: (i) identification of defined MHC binding motifs for the major HLA alleles; (ii) selection of peptide sequences from putative TAA that contain these motifs and measurement of their capacity to bind to purified MHC molecules; and (iii) determination of which MHC-binding peptides can elicit CTL capable of recognizing tumor cells.

Identification of MHC binding motifs

An important factor to consider in the identification of TAA is whether a peptide can bind to a specific MHC allele since MHC binding is a prerequisite for immunogenicity. Peptide binding to an individual MHC molecule depends on the specific sequence of the peptide. Analysis of the sequence patterns of peptides that bind to MHC molecules in humans and mice has revealed the presence of primary anchor residues, in humans usually at positions 2 and at the carboxy-terminal end. MHC molecules are extremely polymorphic, and theoretically each allelic type will bind different sets of peptides (different alleles of the MHC tend to vary in those residues that form part of the peptide binding pockets).[5] The MHC binding motifs for HLA-A1, -A2, -A3, -A11, -A24, -B7 (as well as others) have been reported (Table 2).[36-38] By identifying a set of tumor-associated peptides that can bind to these six HLA alleles mentioned above, one can offer coverage to the majority of the human population.

Selection from TAA of motif-containing peptides and measurement of their MHC binding capacity

The next step in the identification of anti-tumor CTL epitopes is to study a known or a potential TAA sequence for the presence of peptides containing class I MHC binding motifs. Once the TAA have been screened for sequences that contain MHC binding motifs, synthetic peptides representing these sequences are synthesized and tested for their capacity to bind purified HLA molecules.[39] This point is critical because although most (if not all) MHC binding peptides contain binding motifs, only about

Table 2. HLA class I specific binding motifs

HLA-molecule	Anchor position	Preferred* aa	Tolerated† aa	Average frequency‡
A1§	2	TSM		
	3	DE	AS	11.9
	9 (10)	Y		
A2.1	2	LM	IVAT	43.1
	9 (10)	VLI	AMT	
A3.2	2	LMVISATF	CGD	12.0
	9 (10)	KYR[HF]	A	
A11	2	VTMLISAGN	CDF	14.8
	9 (10)	K	R[H]Y	
A24	2	YF[W]	M	28.7
	9 (10)	FLIW		
B7	2	P		12.3
	9 (10)	FLVIM	WYA	

*Aa in bold indicate residues identified by pool sequencing. Aa in plain type indicate residues identified by an analysis in the binding capacity of poly A containing peptides with different aa at the anchor positions. Aa enclosed in brackets are speculated to bind based on chemical similarity to known preferred or tolerated residues.
†Binding motifs are composed of either two preferred anchors or one preferred and one tolerated anchor.
‡Average for Caucasian, Black, Chinese, japanese and Hispanic populations. Data calculated taking information from 'The Central Data Analysis Committee. Allele Frequencies, The Data Book of the 11th Intl. Histocompatibility Workshop, Yokohama 1991'.
§For HLA-A1 anchor positions are: 2 and 9 (10) or 3 and 9 (10).

one third of motif-containing peptides can bind effectively to MHC molecules.[38,39] We have developed quantitative high throughput binding assays that allow us to screen a large number of motif-containing peptides from TAA and determine their binding affinity to several different HLA alleles.[40,41] These assays measure the concentration of peptide (nM) required to inhibit 50% of the binding of a standard radiolabeled peptide to purified soluble HLA molecules. Under the conditions of the assay this figure allows us to approximate the binding constant (Kd) of the interaction and to classify peptides into 'high', (< 50 nm) 'intermediate' (50–500 nM), 'low' (500–5000 nM) on non MHC binders (> 5000 nM).

Testing MHC binding peptides from TAA for CTL immunogenicity

The last step in the epitope identification process is to determine whether the peptides that have been identified as MHC binders (high, intermediate and low binding affinity binders) can induce anti-tumor CTL responses. Primary CTL induction using synthetic peptides can be done either *in vivo* using appropriate HLA transgenic mice[42] or *in vitro* with human peripheral blood mononuclear cells (PBMC) from appropriate HLA-typed individuals.[43,44]

Experiments in HLA-A2.1 transgenic mice have demonstrated that only those peptides that bind to HLA-A2.1 with a high or intermediate affinity (IC_{50} < 500 nM) are capable of eliciting a CTL response following immunization[42] and in fact epitopes for some TAA have been identified by this procedure.[45] There are two main limitations to this approach: (1) mouse strains are not available for several of the HLA alleles of interest, and (2) the TcR repertoire specific for TAA derived from tissue-specific proteins may differ in mice and humans due to tolerance at the T-cell level.

To deal with these potential problems, we have also developed an in-vitro protocol to elicit anti-tumor CTL using primary lymphocyte cultures stimulated with MHC binding peptides selected from tumor-associated proteins.[43] Responder cells, which are enriched for CTL precursors ($CD8^+$ T cells), are incubated in the presence of IL-7 with autologous professional APC pulsed with potential CTL epitopes. The two techniques described above have allowed the identification of several CTL epitopes derived from various TAA sequences.

For example, using lymphocytes from a normal individual we induced an in-vitro primary CTL response to a high affinity HLA-A1-binding peptide from the *MAGE-3* antigen. The resulting *MAGE-3*-specific CTL were capable of killing HLA-A1 tumor target cells (melanomas, prostate and breast tumors)

Table 3. Identification of HLA-A2-restricted CTL epitopes from HPV-16

HPV-16 Peptide protein (Position)	Sequence*	HLA-A2.1 Binding IC^{50} (nM)†	In-vitro CTL (Primary)‡	In-vitro CTL (Transgenics)§
E6 (18–26)	KLPQLCTEL	328	0/6	0/6
E6 (29–38)	TIHDIILECV	494	1/6	8/11
E6 (52–60)	FAFRDLCIV	130	1/7	0/3
E7 (7–15)	TLHEYMLDL	188	1/7	0/6
E7 (11–20)	YMDLQPETT	46	4/7	9/12
E7 (82–90)	LLMGTLGIV	8	3/8	9/12
E7 (86–93)	TLGIVCPI	7	6/9	15/15
E6 (7–15)¶	AMFQDPQER	1818	0/6	0/3

*Sequences shown in bold represent the two peptides chosen as components of a therapeutic vaccine to treat cervical carcinoma (see text).
†MHC binding performed as described.[38,39,41]
‡Numbers represent the experiments where positive CTL were induced/total number of experiments.[45]
§Number of mice where CTL response was positive/total number of mice tested.[45]
¶Negative control peptide (non MHC binder).

that expressed the *MAGE-3* product.[43] Confirming the relevancy of this approach is the fact that independently Boon identified the same epitope by screening an expression library with a CTL clone derived from a melanoma patient.[46] A peptide-based therapeutic vaccine containing this CTL epitope from *MAGE-3* is currently being tested in patients suffering from malignant melanoma.

In another application of the methodology described herein, CTL epitopes from the sequence of the early proteins human papillomavirus type 16 (HPV-16) were identified with the goal of developing a vaccine to treat (or prevent) cervical adenocarcinoma, which is the malignant disease in humans that has been most clearly associated with a viral infection.[32] The sequences of the E6 and E7 proteins of HPV-16 were analysed for the presence of peptides capable of binding to HLA-A2.1 molecules, and the corresponding peptides were tested for their capacity to induce CTL responses.[41,45] These proteins were chosen since their genes are almost always found in tumor samples and they have been shown to have oncogenic properties.[32] Several of the HLA-A2.1-binding peptides derived from these proteins elicited CTL responses both *in vitro* using human lymphocytes and *in vivo* in HLA-A2.1 transgenic mice (Table 3).[45] Two of the peptides identified in these studies are included in an immunotherapeutic vaccine that is currently being tested in women with advanced cervical carcinoma in The Netherlands.

Vaccine development

Various paths can lead to the identification of tumor-associated CTL epitopes, as described above. Next, this information is applied to the production of immunotherapeutics to treat or prevent the occurrence of specific tumors. Amongst the many challenges to the development of effective anti-tumor therapies are: (1) poor immunogenicity of unmodified synthetic peptides; (2) possible tolerance to self proteins; and (3) the large tumor burdens associated with advanced disease, which may induce a state of immunosuppression in the patient and decrease CTL effectiveness. In this section we will discuss some strategies which are currently being applied to overcome these obstacles, and why we believe that the use of synthetic peptide-based vaccines may be advantageous over protein or DNA based vaccines.

Enhancing peptide immunogenicity

In general, when CTL peptides are administered alone (in saline) they fail to induce immune responses. Several adjuvants including Freund's Incomplete Adjuvant (IFA), detergent-based and liposome-based adjuvants have been reported to enhance the capacity of peptides and proteins to induce CTL. Another more successful method (at least in our hands), has been to conjugate lipid tails (palmitic acid) to the peptide itself. Synthetic lipopeptides have been shown to induce strong CTL responses both in humans and animals.[47] Since most CTL responses appear to be regulated by helper T cells, epitopes that stimulate these T cells have also be incorporated into the synthetic peptide vaccines in order to increase their CTL-inducing potency.

Overcoming immune tolerance

As mentioned above, many potential TAA are derived from normal self proteins that may have induced some state of immunologic tolerance, either centrally in the thymus or in peripheral lymphoid tissue. Perhaps the most compelling reason for choosing a peptide-based vaccine approach over one that uses recombinant proteins or DNA, is the ability to select as immunogens those peptide epitopes against which tolerance has not been established. This advantage has been well documented in the study of the class II MHC restricted responses to several autologous or foreign antigens to which tolerance was induced.[48-50] These studies indicate that whereas animals are fully tolerant when the whole protein is used as an immunogen, certain peptides of the protein are capable of inducing immune responses. In dissecting which peptides were immunogenic and which were not, it was found that the immunodominant peptides (in a non-tolerant animal) were the peptides to which tolerance had been induced, but that sub-dominant and 'cryptic' epitopes (that usually do not elicit responses when the whole protein is used as an immunogen) have not induced a state of tolerance and were therefore immunogenic when used as peptide antigens. *The ability, with the synthetic peptide approach, to specifically identify those epitopes of potential TAA to which the individual is capable of mounting an immune response gives this approach a tremendous conceptual advantage over other approaches that use whole protein or their genes as immunogens.*

Since most immunodominant epitopes are high affinity MHC binders,[39] one strategy to help identify sub-dominant epitopes is to concentrate on 'intermediate to low' binding peptides as potential immunogens. In support of this line of thought is the interesting observation that many of the tumor-associated CTL epitopes derived from self TAA that have been identified in tumor-bearing patients, bind with an intermediate affinity to class I MHC molecules (Table 1). This is in contrast to the observation that anti-viral CTL responses in general involve the recognition of the high affinity MHC binding peptides[39,42,45] (e.g. HPV in Table 3).

Immunotherapies for early versus advanced disease

Cancer immunotherapies are likely to be more effective in patients with little or no apparent signs of disease than in those with large tumor masses and multiple metastases. Thus, the ideal scenario would be to utilize CTL-inducing peptide vaccines as adjuvant treatments after resection of primary tumors prior to the appearance of recurrent of metastatic disease. Furthermore, whereas healthy individuals who are at high risk to develop particular forms of cancer (because of genetic or environmental factors) could be treated prophylactically with a vaccine derived from self-protein sequences only after safety concerns regarding potential autoimmune pathological responses are addressed; vaccines derived from oncogenic viral sequences might pose fewer potential hazards when administered to healthy tumor-susceptible individuals.

The best hope of efficient antigen-specific immunotherapies for patients with advanced cancer may be adoptive transfer of TAA-specific CTL.[2,51] These CTL may be more efficiently produced *in vitro* using synthetic peptides, the appropriate professional APC and the optimal combination of cytokines than the protocols currently in place to expand TIL. This type of ex-vivo expanded tumor-specific CTL therapy will make it possible to overcome potential immunosuppressed states of advanced cancer patients, and toxic effects of high doses of systemically administered lymphokines, which can be safely used in tissue culture during the expansion of the CTL cultures.

Other advantages of peptides over proteins and DNA as cancer vaccines

In addition to some of the points made above with respect to peptide based vaccines, it is worth mentioning some of the additional properties that make them more appealing than whole proteins or recombinant DNA-based vaccines for their use to treat or prevent cancer: (1) by using peptides representing single CTL epitopes it is possible to focus the immune response (e.g. conserved oncogenic viral epitopes, subdominant CTL determinants); (2) avoidance of sequences in the TAA with immunosuppressing activity, or with high degree of homology with other proteins in normal tissues (avoiding the degree of unwanted autoreactive responses); (3) it may be simpler, safer and cost-effective to construct multi-epitope vaccines by combining peptides representing CTL epitopes derived from different TAA (e.g. CEA,[52] $p185^{HER-2}$, *MAGE-3* for breast cancer; MART-1, gp100/pMel17, *MAGE-3* for melanoma; PSA and PAP for prostate cancer) than producing and mixing the entire sets of proteins or individual recombinant viruses.

In conclusion, it is evident that several challenges exist in the design of immunotherapeutics for cancer.

Here we have described some of the approaches that are currently being utilized to identify TAA, to define the anti-tumor CTL epitopes in these molecules, and finally to utilize this information for the design and production of vaccines to treat specific malignancies. Some of these vaccines are being tested in the clinic, the validity of the approach should be known in the near future.

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